

# Gastroprotective effects of methanol extract of *Eremomastax speciosa* leaf harvested in Southern part of Nigeria in rat

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## Abstract

Gastric ulcer is one of the common cause of hospital consultation with an increasing prevalence worldwide and it is traditionally managed with herbal medicine in the developing countries. This study investigated the gastroprotective effects of methanol extract of *Eremomastax speciosa* leaf in rats. Cold maceration in 80% methanol was adopted during extract preparation while gas chromatography mass spectroscopy (GC-MS) was employed in the phytochemical analysis. The doses of 25, 50 and 100 mg/kg *E. speciosa* were used on ethanol- and indomethacin-induced gastric ulcer models in rats. The Shay rat method was used to determine the effects of ESE on gastric acidity while the anticholinergic and antihistaminic activities were investigated on isolated rabbit jejunum *ex vivo*. The GC-MS analysis identified six bioactive compounds. Both ESE and cimetidine significantly ( $p < 0.05$ ) reduced the severity of indomethacin- and ethanol-induced gastric injuries and gastric acid contents in Shay rats. The extract elicited concentration-dependent relaxation of isolated rabbit jejunum and reduced the contraction induced by both acetylcholine and histamine in the same tissue. The findings showed that ESE protected the rats against chemical-induced gastric ulcer through anticholinergic and antihistaminic mechanisms.

**Keywords:** Acetylcholine, antiulcer, *Eremomastax speciosa*, histamine, indomethacin

## Introduction

Gastric ulcer is one of the common cause of hospital consultation with an increasing prevalence worldwide (1–3). The increase in prevalence is attributed to indiscriminate use of non-steroidal anti-inflammatory drugs (NSAIDs), alcoholism, smoking and stress (4). The NSAIDs like Aspirin, inhibit cyclooxygenase (COX) activity and decrease prostaglandins production (5). Prostaglandin deficiency disrupts the normal protective mechanism that guard the gastric mucosa against ulceration (6–8). Besides neutrophil-dependent induced gastric ulcers by NSAIDs, other contributors to the pathology include cigarette smoking, alcohol consumption and *Helicobacter pylori* (2, 9). In clinical practice, gastric ulcer is usually treated with antacids, H<sub>2</sub>-receptor antagonists, prostaglandin analogues, proton pump inhibitors, and antibiotics (1). In developing countries about 80% of the populace explore medicinal herbs in the treatment of their ailment (10). *Eremomastax speciosa* is one of the herbs used in the treatment of stomach ulcer (11).

*Eremomastax speciosa* (Hochst) Cufod (Family Acanthaceae) normally grows as weed in the wild, although now cultivated for its medicinal properties in different parts of Africa (12), particularly in Akwa Ibom State, Nigeria (13,14) and Anglophone Cameroun (15). *Eremomastax speciosa* is polymorphous shrub with characteristic quadrangular stem and violet coloured leaves (11). It is widely used for its anti-anemic property (16) which has earned the plant, local names, such as “blood plant” or “*pang nyemshe*” by natives of Cameroun; “golden seal” or “African blood tonic plant;” in Nigeria, and “*edem ididout*” by the Ibibios

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in Akwa Ibom State, Nigeria (12). It is also used by the natives in the treatment of stomach pains (oral communiation). The anti-inflammatory, antioxidant, anti-nociceptive, anti-anemic, and anti-secretory activities of different parts of *E. speciosa* have been reported (11,12,17,18). However, there is dearth of information on the gastroprotective abilities of leaf extract of *E. speciosa* harvested in Nigeria. Therefore, this study evaluated the gastroprotective effects of methanol extract of *Eremomastax speciosa* leaf harvested in Nigeria.

## Materials and Methods

### Harvest and authentication of plant material

The leaves of *E. speciosa* were harvested from Ikot-Ekwere Itam in Itu, Akwa-Ibom State, Nigeria and was identified by a botanist, Mr. Okon Etefia. A voucher sample was kept in the departmental herbarium with reference number MOUAU/VPP/2015/15.

### Extract preparation

The leaves were washed under running tap water, and then air dried on top of the laboratory bench. The dried and crispy plant leaves were pulverized into a coarse powder using a laboratory blender. The coarsely pulverized leaves of *E. speciosa* (500 g) were extracted by cold maceration in 80:20 methanol (Sigma Aldrich, Germany) in distilled water for 48 h at room temperature with intermittent agitations every 3 h. Thereafter, the extract was filtered using Whatmann No. 1 filter paper and concentrated at 40°C in a hot air oven. The resulting extract was designated, *Eremomastax speciosa* extract (ESE) and stored in a refrigerator at 4°C until required.

### Gas chromatography-mass spectra (GC-MS) analysis

Analysis was conducted using GC-MS (GCMS-QP2010 PLUS SHIMADZU, JAPAN) equipped with a DB-5MS column (30 m × 0.25 mm i.d., 0.25 µm film thickness, J & W Scientific, Folsom, CA). The initial oven temperature was 60 °C. Helium at 1.0 mL/min was used as the carrier gas. The eluents were introduced directly into the MS through a transfer line maintained at 250 °C. The ionization voltage was 70 eV and ion source temperature was 230 °C while the scan range was 41- 450 amu. The components were identified by comparing their retention times with the National Institute of Standards and Technology (NIST, ver. 2.0, 2008) mass spectral library database.

### Experimental animals

A total of seventy-five (75) albino Wistar rats of both sexes and one rabbit sourced from the laboratory animal unit of Department of Veterinary Physiology and Pharmacology, Michael Okpara University of Agriculture, Umudike were used for the study. The animals were housed in wire-mesh rodent cages and had unrestricted access to pelleted rat feed (Vital Feed) and water except when fasting were required. They were fasted before the experiments; food (16 h) and water (2 h) prior to drug or ESE administration. The experimental protocol was approval by the Michael Okpara University of Agriculture,

Umudike Animal Use Ethics Committee, and all animals were humanely handled as stipulated in the Guide for the Care and Use of Laboratory Animals of National Research Council. (19).

### Experimental design

#### *Indomethacin-induced gastric ulcer model*

The gastroprotective effects of ESE against indomethacin-induced gastric ulcers were studied according to the method of Inas et al (23). Briefly, Wistar rats of both sexes (n=25) were weighed and randomly assigned to five groups (A-E).

Group A: received distilled water 5.0 ml/kg (control)

Group B: received cimetidine 100 mg/kg (reference standard)

Group C: received ESE 25 mg/kg

Group D: received ESE 50 mg/kg

Group E: received ESE 100 mg/kg

The doses used in this study were based on previous studies (18). All doses of ESE, cimetidine and distilled water were administered orally with the aid of a gastric gavage. One hour (1 h) post treatment, the rats were given indomethacin (Romacid® Jiangsu, China) (40 mg/kg body weight) *per os*. Four hours (4 h) post indomethacin administration the rats were sacrificed through cervical dislocation, and the stomachs were removed, cut open along greater curvature and were washed under slow running tap water. Ulcer lesions were counted with the aid of magnifying lenses (×10 magnification), and scored on the basis of intensity as follow; 0 = no ulcer, 1 = superficial ulcer, 2 = deep ulcers, 3 = perforations. Thereafter, ulcer index and percentage inhibition were calculated as follows:

$$\text{Ulcer index } (U_i) = (U_N + U_s + U_p) \times 1/10$$

Where,  $U_N$  = average number of ulcer per animal,  $U_s$  = average severity score,  $U_p$  = percentage of animal with ulcer and  $U_i$  = ulcer index. The percentage ulcer inhibition (PUI), was determined using the formula:

$$\text{PUI} = \frac{\text{UI (control)} - \text{UI (Treatment)}}{\text{UI (control)}} \times \frac{100}{1}$$

#### *Ethanol-induced gastric ulcer*

The gastroprotective effect ESE against ethanol-induced ulcer model was studied according to the modified method of Silva et al. (24). Twenty five (25) Wistar rats were randomly assigned to five (A-E) groups (n = 5), as described in previous experiment, however, misoprostol (Zahidi Enterprise, India) 50 µg/kg body weight, was used as the reference drug. 1 h post treatment, all the animals were given 1 ml of ethanol (Sigma Aldrich, Germany) via oral gavage. Two hours later all the animals were sacrificed through cervical dislocation, and their stomach were removed and cut open along the greater curvature. The ulcer lesions were observed as previously described, and UI and PUI, calculated as aforementioned under indomethacin-induced ulceration.

#### *Pyloric ligation method*

The modified method of Shay et al., (25) as presented in Sharma et al. (26) was adopted for this investigation with slight

modifications. Adult Wistar rats were grouped into 5 groups (A-E, n = 5 each) and food was removed for 48 h, in a modified metal cage to discourage coprophagy. Groups A – E were orally treated with ESE and controls as previously mentioned under indomethacin-induced ulceration. Immediately after 1 h of oral treatments, the rats were anaesthetized by intraperitoneal administration of thiopentone sodium (30 mg/kg body weight). A laparotomy was carried out, and the pyloric sphincter loosely ligated to avoid occluding blood vessels. The stomach and abdominal contents were immediately sutured carefully in 2 layers of interrupted suture pattern using silk. They were placed in improvised recovery tubes (2-inch plastic pipe). The animals were starved of water post-operation. Sixteen hours post operation, each rat was sacrificed through cervical dislocation and eviscerated, the stomach exteriorized, and entire stomach content collected into a calibrated tube, to determine gastric juice volume, then centrifuged at 3000 rpm for 5 min and the supernatant titrated against 0.1 M NaOH to determine acidity.

#### Histopathologic examination

Sample stomach tissues from test rats of indomethacin-induced ulcer models were collected and fixed in 10% formal saline. Thereafter, sectioned and hematoxylin and eosin (H & E)-stained stomach tissues were viewed under digital light microscope (10).

#### Assessment of the effects of ESE on isolated rabbit jejunum ex vivo

The effect of ESE on intestinal smooth muscle contraction was evaluated using isolated rabbit jejunum with the aid of a physiograph as described by Ijioma et al (24). ESE was tested at final bath concentration in the ranges of 57 – 456 µg/ml in the absence and presence of acetylcholine (0.029 µg/ml) and histamine (0.29 µg/ml). The final bath concentration was determined using the formula below:

Final bath concentration (FBC) =

$$\frac{\text{test concentration} \times \text{volume used}}{\text{bath volume}}$$

#### Statistical analysis

Data obtained were presented as mean ± SEM and analysed using one-way analysis of variance (ANOVA) and post-hoc comparisons were carried out using either Dunnett's t-test or the Newman-Keuls test (where appropriate) on GraphPad Prism version 4.05. Values of *P* < 0.05 were considered significant in the study.

## Results

#### Chemical composition of ESE as identified by the GC-MS

Six (6) compounds were identified during the GC-MS analysis of ESE. The GC-MS chromatogram shown 6 peaks (Fig. 1). The identified compounds are benzenesulfonyl chloride (40.63%), benzopyran-4-one, 5,7-dihydroxy-2-phenyl- (21.25%), benzenesulfonic acid, methyl ester (15.63%), 2(5H)-furanone, 4-methoxy-5-phenyl- (12.50%), 4,5-dimethylthiazole (4.38%) and p-chlorobenzenesulfonyl chloride (5.63%) (Table 1).

#### Effects of ESE against indomethacin-induced ulcer

Oral administration of the ESE (25 mg/kg) and cimetidine (100 mg/kg) produced significantly (*P* < 0.05) decreased ulcer score, ulcer severity and ulcer index in test rats compared with the control group (Table 2).

#### Effects of ESE against ethanol-induced gastric ulcer

All the doses of ESE (25, 50 and 100 mg/kg) and misoprostol 50 µg/kg produced significantly (*P* < 0.05) decreased ulcer index compared with distilled water treated rats (control). The inhibition of ulcer index in treated groups did not show dose-dependent effect (Table 2). Although, the standard reference, misoprostol significantly (*P* < 0.05) decreased the ulcer score

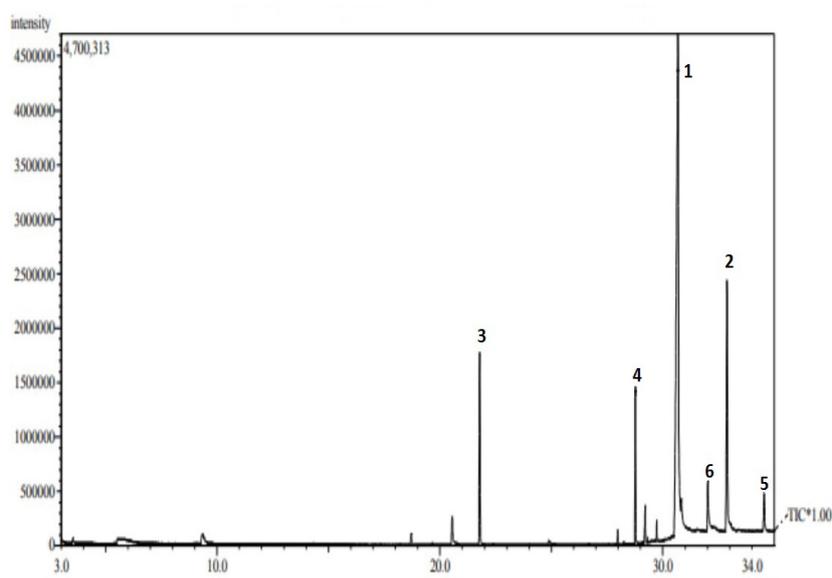


Figure 1. GC-MS chromatogram of ESE.

1 = benzenesulfonyl chloride, 2 = benzopyran-4-one, 5,7-dihydroxy-2-phenyl-, 3 = benzenesulfonic acid, methyl ester, 4 = 2(5H)-furanone, 4-methoxy-5-phenyl-, 5 = 4,5-dimethylthiazole, 6 = p-chlorobenzenesulfonyl chloride.

**Table 1.** Chemical composition of ESE as identified by the GC-MS

S/N	RT (min)	%	MW (g)	MF	Chemical name
1	30.60	40.63	176	C <sub>6</sub> H <sub>5</sub> ClO <sub>2</sub> S	Benzenesulfonyl chloride
2	33.00	21.25	254	C <sub>15</sub> H <sub>10</sub> O <sub>4</sub>	Benzopyran-4-one, 5,7-dihydroxy-2-phenyl-
3	21.80	15.63	172	C <sub>7</sub> H <sub>8</sub> O <sub>3</sub> S	Benzenesulfonic acid, methyl ester
4	28.80	12.50	190	C <sub>11</sub> H <sub>10</sub> O <sub>3</sub>	2(5H)-Furanone, 4-methoxy-5-phenyl-
5	34.50	4.38	113	C <sub>5</sub> H <sub>7</sub> NS	4,5-Dimethylthiazole
6	32.00	5.63	210	C <sub>6</sub> H <sub>4</sub> Cl <sub>2</sub> O <sub>2</sub> S	p-Chlorobenzenesulfonyl chloride

RT = retention time, % = percentage composition, MW = molecular weight, MF = molecular formula

**Table 2.** Effects of ESE against indomethacin-induced gastric ulcer

Group	Ulcer score	Ulcer severity	Ulcer index	% inhibition
Distilled water, 5 ml/kg	9.80 ± 1.46	16.00 ± 0.84	12.58 ± 0.07	-
Cimetidine, 100 mg/kg	5.80 ± 1.11*	10.80 ± 2.82	11.66 ± 0.32*	7.31
ESE, 25 mg/kg	4.00 ± 0.82*	6.50 ± 1.55*	11.05 ± 0.18*	12.16
ESE, 50 mg/kg	8.50 ± 1.20	14.25 ± 1.80	12.28 ± 0.29	2.38
ESE, 100 mg/kg	8.00 ± 2.31	15.67 ± 5.93	12.37 ± 0.58	1.67

\*p < 0.05 compared with distilled water treated rats, ESE = *Eremomastax speciosa* extract

**Table 3.** Effects of ESE on ethanol-induced ulcer

Group	Ulcer score	Ulcer severity	Ulcer index	% inhibition
Distilled water, 5 ml/kg	8.33 ± 1.65	17.33 ± 3.94	12.57 ± 0.55	-
Misoprostol, 50 µg/kg	0.50 ± 0.29*	1.00 ± 0.71*	5.15 ± 0.07*	59.03
ESE, 25 mg/kg	3.75 ± 2.25	9.00 ± 5.93	6.28 ± 0.63*	50.04
ESE, 50 mg/kg	7.67 ± 1.17	17.67 ± 3.15	8.53 ± 0.40*	32.14
ESE, 100 mg/kg	5.00 ± 2.41	8.40 ± 3.61	7.34 ± 0.49*	41.61

\*p < 0.05 compared with distilled water treated rats, ESE = *Eremomastax speciosa* extract

**Table 4.** Effect of ESE on total gastric acidity

Group	Total gastric acidity (mole/L)	% reduction
Distilled water, 5 ml/kg	0.08 ± 0.00	-
Cimetidine, 100 mg/kg	0.03 ± 0.01*	62.5
ESE, 25 mg/kg	0.06 ± 0.00	25
ESE, 50 mg/kg	0.04 ± 0.01*	50
ESE, 100 mg/kg	0.07 ± 0.01	12.5

\*p < 0.05 when compared with distilled water treated group, ESE = *Eremomastax speciosa* extract

and severity, the extract at all doses did not show any significant ( $p > 0.05$ ) decrease in ulcer score or severity (Table 3).

#### Effect of ESE on total gastric acidity

The extract, ESE (50 mg/kg) elicited significantly ( $P < 0.05$ ) decreased total gastric acidity, while ESE 25 and 100 mg/kg as well as cimetidine (100 mg/kg), produced no significant ( $P > 0.05$ ) reduction in total gastric acidity compared with the control group. The highest dose of ESE (100 mg/kg) used in the study produced comparable percentage reduction in total gastric acidity (Table 4).

#### Histopathologic examination

Fig. 2 (A–E) show representative photomicrographs of the histopathological sections of stomach from indomethacin-induced gastric ulcers of groups A to E. These sections of the stomach indicate the severity of the ulcers (indicated using arrows). The distilled water treated (A), and ESE (100 mg/kg; E) showed increased numbers as well as deeper gastric ulcerations when compared to cimetidine (100 mg/kg; B) or ESE (25 and 50 mg/kg; C and D respectively) treated groups (Fig. 2).

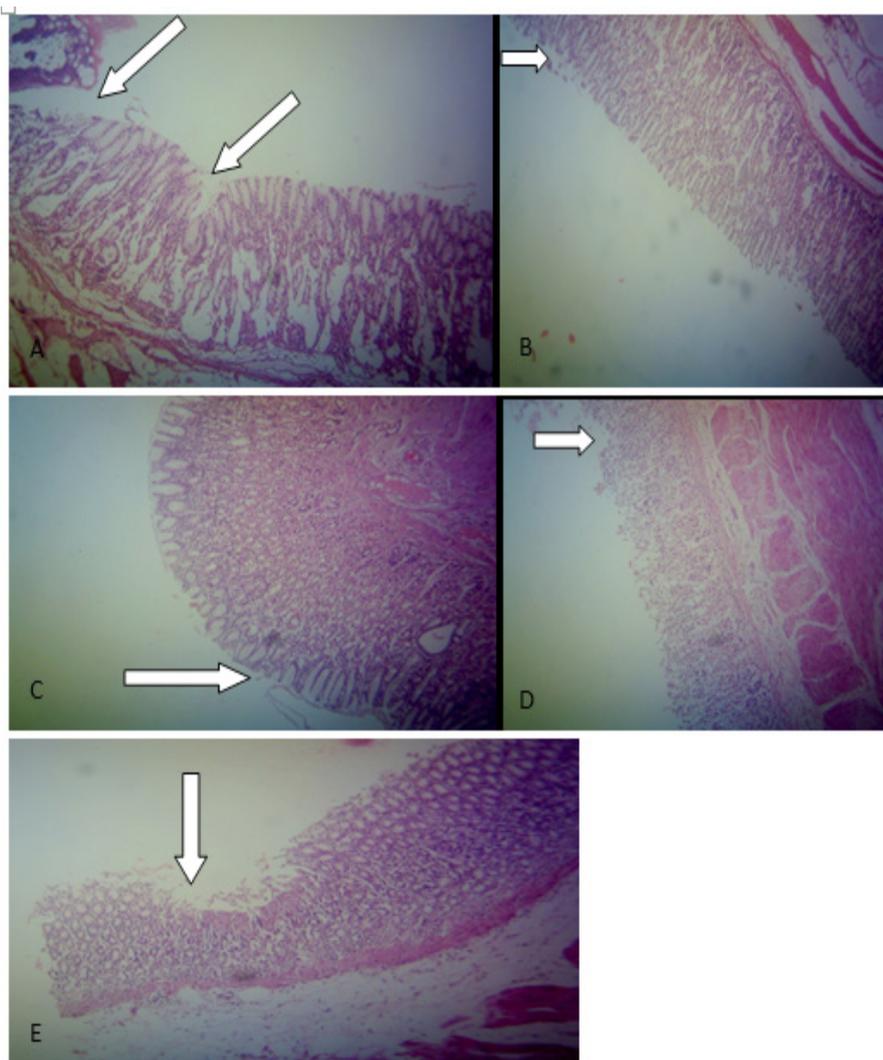
#### Effect of ESE on isolated rabbit jejunum

All concentrations of ESE (57 – 456  $\mu\text{g/mL}$ ) used in this study produced a concentration-dependent relaxation of the intrinsic contraction of the isolated rabbit jejunum (Fig. 3). ESE also caused inhibition of both acetylcholine- and histamine-induced increase in intrinsic contraction of isolated jejunum (Figs. 4-6).

#### Discussion

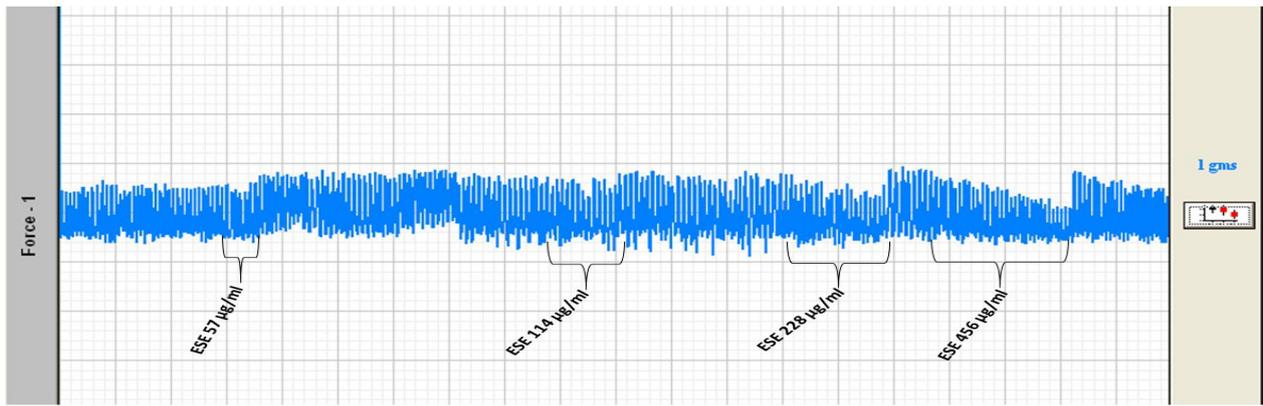
This study evaluated the gastroprotective effects of the methanol extract of *Eremomastax speciosa* leaf on experimentally induced ulcers in rats. The *E. speciosa* extracts (ESE) produced gastroprotective effects in the ulcer models tested as well as elicited anticholinergic and antihistaminic activities. The GC-MS analysis of ESE identified six bioactive compounds. The gastroprotective activities of *E. speciosa* was attributed to the presence of benzopyran-4-one, 5,7-dihydroxy-2-phenyl- (chrysin).

Prostaglandin stimulates increased bicarbonate and mucus synthesis and enhance mucosal blood flow, hence, maintaining mucosal repair and integrity of the mucosa (25). Suppressed

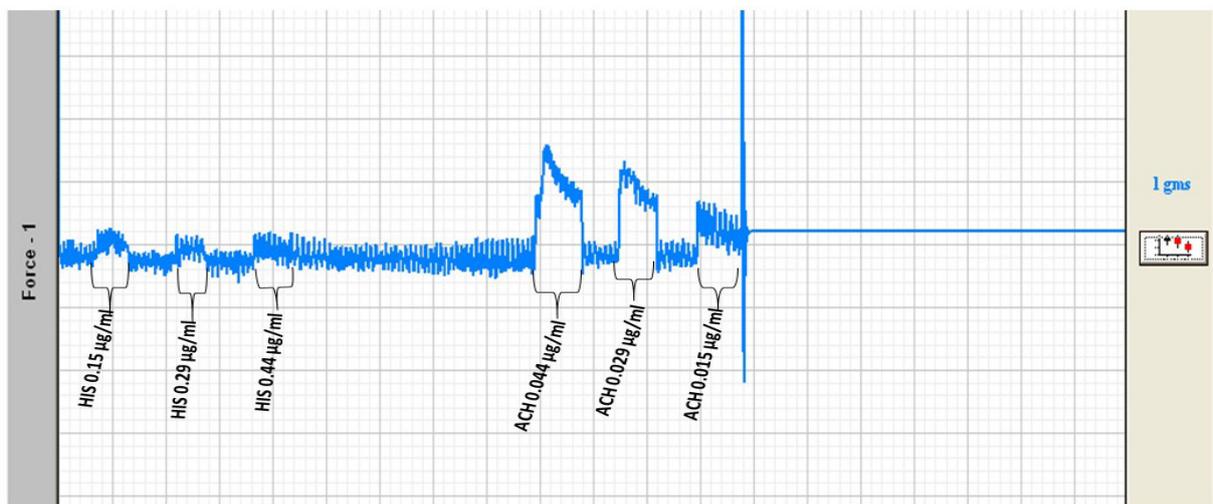


**Figure 2.** Stomach sections of effects of ESE against indomethacin-induced gastric ulcer.

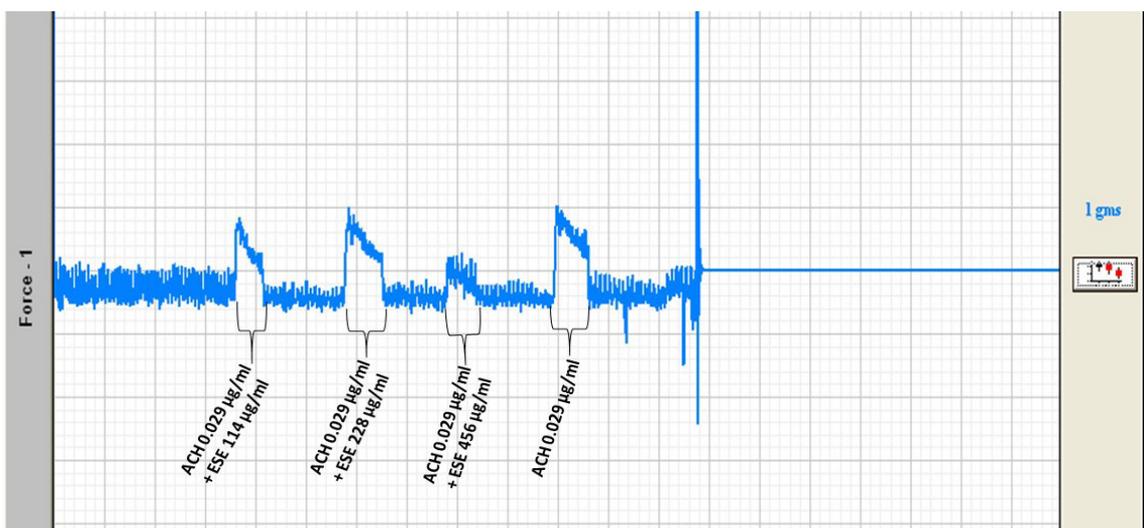
Arrows indicate points of gastric ulceration. Plates A- E represents various treatments; groups A = distilled water 5.0 ml/kg, B = cimetidine 100 mg/kg, C = ESE 25 mg/kg, D = ESE 50 mg/kg, E = ESE 100 mg/kg (x 100 magnification).



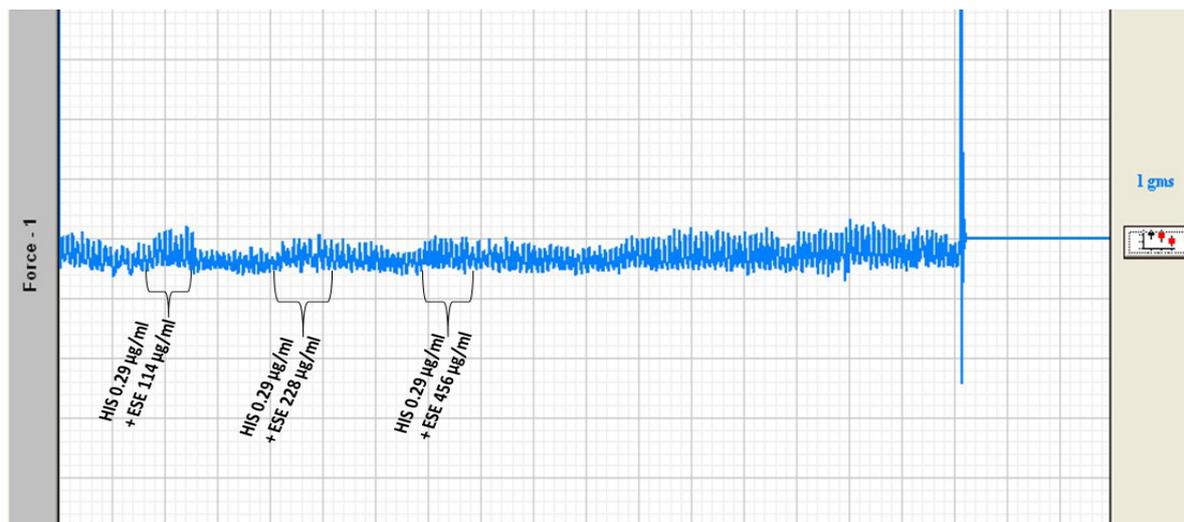
**Figure 3.** The relaxant effects of ESE on isolated rabbit jejunum *ex vivo*.  
 ESE = *Eremomastax speciosa* extract, final bath concentration of ESE ranged between 57 – 456 µg/mL.



**Figure 4.** Concentration-dependent effects of histamine and acetylcholine on the intrinsic contraction in isolated rabbit jejunum *ex vivo*.  
 HIS = histamine, ACH = acetylcholine, the final bath concentration of histamine was between 0.15 – 0.44 µg/mL while the final bath concentration of acetylcholine was between 0.015 – 0.044 µg/mL.



**Figure 5.** The inhibitory effects of ESE on acetylcholine-induced increased intrinsic contraction of isolated rabbit jejunum *ex vivo*.  
 ACH = acetylcholine, ESE = *Eremomastax speciosa* extract, the final bath concentration of acetylcholine was 0.029 µg/mL while the final bath concentration of ESE ranged between 114 – 456 µg/mL.



**Figure 6.** The inhibitory effects of ESE on histamine-induced increased intrinsic contraction of isolated rabbit jejunum *ex vivo*. HIS = histamine, ESE = *Eremomastax speciosa* extract, the final bath concentration of histamine was 0.29 µg/mL while the final bath concentration of ESE ranged between 114 – 456 µg/mL.

prostaglandins and elevated free radical levels predispose to stomach mucosal injury and duodenal ulceration (26,27). Indomethacin and ethanol induces gastric ulcers via the inhibition of COX, a pivotal enzyme for prostaglandin biosynthesis from arachidonic acid as well as the formation of free radicals (5,20,27–29). Antioxidants prevent oxidative damage of cell membranes and enhance ulcer wound healing (30). The anti-ulcer activities of ESE could be akin to enhanced prostaglandin synthesis and its antioxidant activity (11,18). We previously associated, as a probable mechanism of anti-nociception effects of *E. speciosa*, to the inhibition of cyclooxygenase required to catalyze the synthesis of prostaglandins (18). Other researchers have previously reported on the anti-ulcer and anti-nociceptive activities of *Melastoma malabathricum*, *Maytenus ilicifolia* and *Piper aleyreanum* (31–33). Our findings are corroborated in earlier reports on the antiulcer activities of *Barleria gibsoni* and *Terminalia chebula* fruit (23,30).

The percentage ulcer inhibition of extract (25 mg/kg) was comparable to misoprostol (50 µg/kg) in the ethanol-induced ulcer model. Misoprostol is a prostaglandin analogue that stimulate mucus and bicarbonate production, inhibit gastric acid secretion and cause vasodilation which shield the stomach mucosa against damage (34). This suggests that both ESE and misoprostol conferred cytoprotective effect on gastric mucosa and could be the possible mechanism of action. The mucus-bicarbonate layer create a near neutral microenvironment (pH-7) at the mucus-mucosa interface and also prevent the proteolytic digestion of gastric epithelium by pepsin (35). It was observed that ESE caused dose-dependent increase in the gastric mucus content in the shay rat and ethanol-induced ulcer models, similar to the reference drug (the volume was not quantified; thus data not shown). The increased mucus content suggests that the extract might have influenced the release of chemical mediators that regulate the formation of

gastric mucus such as gastrin, secretin, prostaglandin and acetylcholine (36).

The inhibition of cholinergic and histaminic effects by the extracts may be responsible for the decrease in total gastric contents (36). ESE not only relaxed the jejunum, but also exhibited a concentration-dependent decrease in the magnitude of acetylcholine- and histamine-induced contraction of isolated rabbit jejunum, thus suggesting that ESE possesses anticholinergic and antihistaminic properties (34). The anticholinergic and antihistaminic activities of ESE is in agreement with the report of André Perfusion et al (12). Gastric acid secretion is positively regulated by acetylcholine and histamine receptors in the gastric parietal cell, stimulating the proton pump to release hydrogen (H<sup>+</sup>) ion with concomitant increase in gastric acid content (34, 36), hence the observed decrease in gastric acidity following ESE administration.

The demonstrated gastroprotective potential of *E. speciosa* could be attributed to the presence of Benzopyran-4-one, 5,7-dihydroxy-2-phenyl- (chrysin derivative). Babu et al (37) reported the gastroprotective effects of chrysin and its derivative isolated from *Oroxylum indicum* against pyloric ligation, cold-restrain, ethanol and aspirin induced ulceration models in rats. The findings of this study corroborated the previous reports on the antisecretory and antiulcer activities of *E. speciosa* leaf harvested from Yaounde, Cameroun (11, 12, 38, 39). They differed only in the effective doses of the extracts which could be attributed to the difference in phytochemical composition. The potency of medicinal plants depend on the content and composition of the secondary metabolites such as: saponins, tannins, flavonoids, terpenes, sterols, glycoside and alkaloids which are dependent on climatic factor, soil condition and stage of vegetative growth of the plant at the time of collection (40).

## Conclusion

The finding studies demonstrated that methanol extract of *Eremomastax speciosa* possess potent gastroprotective effects which may be mediated through anticholinergic and antihistaminic mechanisms. This study provide the pharmacological background for the ethnomedicinal use of *E. speciosa* leaf decoction in the treatment of stomach ulcer by the natives.

## Abbreviations

ESE = *Eremomastax speciosa* extract

HIS = histamine

ACH = acetylcholine

GC-MS = Gas chromatography-mass spectroscopy

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## Conflict of interest statement

The authors declare that they have no conflict of interest.

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